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DIAGNOSING SULFUR DEFICIENCY IN SPRING RED WHEAT: PLANT ANALYSIS

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□ *Sulfur (S) availability indicators are necessary for rational fertilizer use. The goals were to assess the predictive capacity of: i) malate:sulfate ratio in leaf; ii) total nitrogen (N):S ratio in aerial biomass; and iii) total N:S ratio in grain. Six experiments were carried out in Argentina for two years. Between 90 and 100% of samples were correctly diagnosed by total N:S ratio during tillering, and critical N:S ratios varied from 14.8:1 to 16:1. At the same time, malate:sulfate ratio diagnosed correctly between the 35 and 65% of the samples. Grains with S deficiency were determined as those with a total S concentration lower than 0.15% and a total N:S ratio higher than 13.3:1. Validation of these new thresholds allowed determining that 77% of the samples were correctly diagnosed. A linear association between grain N:S ratio and N:S in aerial biomass during stem elongation was found ($r^2 = 0.76$–0.78, respectively).*

Keywords: grain analysis, N:S ratio, malate:sulfate ratio, diagnostic methods

INTRODUCTION

Over the last decades sulfur (S) deficiencies have expanded through several regions in the world, including Argentina (Scherer, 2001; Zhao et al., 2002; Echeverría, 2005). Sulfur availability indicators are needed for a rational use of fertilizers and to avoid yield and grain quality losses (Zhao et al., 1999a; Blake Kalff et al., 2002). To diagnose S deficiencies in wheat, methods based on the analysis of soil samples and plant part samples have been proposed, as well as simulations models (Zhao and McGrath, 1994; McGrath and Zhao, 1995; Blake Kalff et al., 2001). Currently, the aforementioned nutrient is routinely applied in areas where a response in previous crops has been observed (Rasmussen and Kresge, 1986).

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Methods based on plant part analysis are preferred, as the S determined by those methods is related with the amount of available S for crops (Melsted et al., 1969). Possible indicators of wheat sulfur status that have been proposed are total S determination (Pinkerton, 1998), sulfate (SO_4; Scaife and Burns, 1986), sulfate: total S ratio (Spencer and Freney, 1980), and glutation (Zhao et al., 1996). However, the critical values determined for these indicators show wide variations depending on crop growth stages, part of the plant analyzed, experiment conditions (field or greenhouse) and the chosen analysis method, all of which limits their use for routine recommendations.

Determination of N:S ratio in grain or in aerial biomass could be a good S availability estimator for crops (Rasmussen et al., 1977; Randall et al., 1981; Withers et al., 1995). For wheat, a joint use of N:S ratio and total S concentration in grain has been suggested (Randall et al., 1981). Carver (2005) determined 82 and 67% of correctly diagnosed samples for wheat and winter barley, respectively. However, when considering only S deficient sites, the predictive capacity of the methodology decreased to 42% for wheat and 0% in barley. Other works determine a lack of accuracy in this methodology as an indicator of the sulfur status of wheat crops (Reussi Calvo et al., 2006a, 2006b). Moreover, Gyori (2005) determined a 0.15% critical concentration of S in grain, whereas Bergmann (1992) suggests the use of a critical N:S ratio in grain lower than the one mentioned by Randall et al. (1981).

Several authors have suggested N:S critical ratios in aerial biomass for controlled conditions (Freney et al., 1978; Zhao et al., 1996) and field (Rasmussen et al., 1977; Blake Kalff et al., 2000). For winter wheat, Spencer and Freney (1980) determined that N:S ratios lower than 16:1 and 19:1 should be the appropriate for the end of tillering and the beginning of stem elongation, respectively. For the period lapsed from the beginning of tillering through the end of stem elongation, other authors obtained critical N:S ratios of 17:1 (Rasmussen et al., 1977). On the other hand, Blake Kalff et al. (2000) reported critical N:S ratios of 15:1 in leaf for the tillering period. However, some papers question the usefulness of this ratio for the first stages of wheat growth, as total S concentration is less sensitive to S availability variations in soil, in relation to sulfate levels in plant (Blake Kalff et al., 2000).

In an experience carried out in the England, Blake Kalff et al. (2004) suggested a determination of malate:sulfate ratio in leaf as S availability indicator for crop. However, the research was executed with soils with low organic matter contents (lower than 2%), pH above 6, and sandy loam textures, all properties typical of soils with a high probability of response to the application of S (Scherer, 2001; Echeverría, 2005), all of which limits the reach of the conclusions obtained by those authors. In a recent work, Carver (2005) determined only 54% of correctly diagnosed samples through use of the malate:sulfate ratio in leaf for winter wheat. The predictive capacity of this methodology in soils with higher content of organic matter, or with finer textures or pH below 6, may be questioned. Currently, there is no research on spring wheat assessing in field conditions the predictive capabilities of total N:S ratio in aerial biomass and grain, and malate:sulfate ratio in leaf for S deficiency diagnosis.

The goal of this work was to evaluate the predictive capacity of malate:sulfate ratio in leaf and total N:S in aerial biomass through the tillering stage of spring wheat. Besides, an additional goal was to determine the behavior of S concentration and total N:S ratio in grain as an indicator of S availability in crop.

MATERIALS AND METHODS

Sites

A total of six experiments were carried out for two years (2006 and 2008) in the southwest of the province of Buenos Aires, Argentina (37° 45' S; 58° 18'W; 130 m above sea level; average annual rainfall of 870 mm and 13.7° C average annual temperature). The first year (2006), four experiments were carried out under rainfed conditions (Sites 1, 2, 3, and 4), whereas in the second year (2008) two experiments were carried out, one under rainfed conditions (Site 5) and the other one under complementary irrigation (Site 6). In Site 6 two 55 mm irrigations were applied in stages Z24 and Z41 according to Zadoks et al. (1974), respectively. Table 1 shows some soil and crops characteristics in each site. The soils were a fine, mixed, thermic Typical Argiudoll (clay: 24%, silt: 33%, and sand: 43%) with a minimum effective depth of 1.5 m.

Experimental Design

Along the first year the experimental design consisted in randomized complete blocks with three repetitions, and the evaluated treatments were four S rates (0, 5, 10 and 20 kg S ha^{-1}), randomly applied at crop sowing. To avoid N as a limiting factor, a rate of 180 kg ha^{-1} was applied (50% of N rate at sowing and 50% at tillering). Along the second year design was in randomized complete blocks with a 4*2 factor arrangement (four N rates and two S rates). Nitrogen rates were 0, 70, 110 and 150 kg ha^{-1}, and S rates were 0 and 40 kg ha^{-1}. Nitrogen and S fertilizers were surface broadcasted at crop sowing. In all experiments, N and S sources were granulated urea (46–0-0) and calcium sulfate (20% S, 16% Ca), respectively. In all experiments a rate of 30 kg P ha^{-1}, as triple super-phosphate, was applied to avoid P deficiencies. The experimental unit size was of 50 m^2 (5 m wide to 10 m long). Weeds, insects, and diseases were controlled using appropriate pesticides.

TABLE 1 Site, crop and soil characteristics

	First year				Second year	
Site	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Years of continuous cropping	15	25	20	20	20	20
Previous crops	Soybean	Sunflower	Soybean	Sunflower	Sunflower	Wheat
Variety	Baguette 21	Baguette 11	Baguette 13	Buck Guapo	Baguette 11	Bio INTA 3000
Tillage	SD	LC	SD	LC	LM	SD
Sowing date	15 June	12 July	18 July	19 July	11 July	30 June
Harvest date	22 Dec.	27 Dec.	26 Dec.	28 Dec.	18 Dec.	16 Dec.
pH (soil water 1:2.5) (0–20 cm)	6.0	5.8	5.8	5.9	6.1	5.8
Organic matter * (g kg^{-1}) (0–20 cm)	60	47	55	51	50	48
$N0_3^-$-N (kg ha^{-1})† (0–60 cm)	85.6	34.6	38.5	64.2	64.0	45.4
P (mg kg^{-1})‡ (0–20 cm)	17.2	22.7	21.1	17.5	21.8	18.8
$S0_4^{-2}$-S (kg ha^{-1})§ (0–60 cm)	28.8	25.5	19.6	29.4	21.5	19.3

SD = no-tillage, LC = conventional tillage, LM = minimal tillage.
*Walkley-Black (Nelson and Sommers, 1996).
†Extracted with KCl (Keeney and Nelson, 1982).
‡Bray 1 (Bray and Kurtz, 1945).
§Extracted with $Ca(H_2PO_4)_2$ (Islam and Bhuiyan, 1998).

Plant Material and Measurements

Plant samples were taken to determine total N and S concentration at stages Z22 (two tillers), Z24 (four tillers), Z31 (one visible node) and Z39 (visible flag leaf ligule) according to Zadoks et al. (1974). For this purpose, plant samples were cut from 0.35 m of six rows selected at random. Samples were dried at 60°C through constant weight and a fraction was ground (0.84 mm mesh) to determine total N and S. Total N and S in aerial biomass was determined by the Dumas dry combustion method at 950 and 1350°C, respectively, using a TruSpec CNS (LECO, St. Joseph, MI, USA) analyzer. At each sampling moment, N:S ratio was obtained by the quotient between total N and S concentration in plant.

To determine the malate:sulfate ratio, leaf samples were taken from 30 plants (last two leaves completely expanded) at stages Z22, Z24, Z31, and Z39. The samples were dried through 3 hours at 105°C (Blake Kalff et al., 2004) and then a fraction of them was grinded. The concentration of malate and sulfate was determined from a watered extract obtained from 50 mg of dried grinded materials. Extraction was done with water at 80°C temperature along 2 hours, later filtered through filter paper (Whatman no. 42) and a 0.2 microns membrane filter. The concentration of these anions was determined with an ion chromatograph (Dionex DX500 with a G50 gradient pump and ED40 electrochemical detector; Dionex, Sunnyvale, CA, USA). The eluent used was 1.8 Mm sodium carbonate and 1.7 mM sodium bicarbonate. Malate:sulfate ratio was estimated by malate peak area and sulfate peak area division following Blake Kalff et al. (2002). The determination of malate and sulfate concentration in leaf was carried on in England by Dr. Mechteld Blake Kalff from the Hill Court Farm Research laboratory.

The harvest was done with an experimental plot harvester over a minimum surface of 7 m^2. Moisture content was determined for each sample of harvested grain and yield was adjusted at 14% moisture. Furthermore, the weight of 1000 grains was determined by drying each sample at 95°C through 24 hs and later weighing out 1000 grains. The number of spikes m^{-2} was obtained counting the spikes from eight 1 m long furrows. In the same way as for aerial biomass, determination of total N and S in grain was estimated by Dumas dry combustion method. Values for total N and S concentration in grain were charted following the methodology proposed by Randall et al. (1981). Furthermore, relative yield was estimated by the quotient between each treatment yield against treatment with 20 and 40 kg S ha^{-1}, for the first and second year, respectively.

To determine the sufficiency limit value of N:S ratio in aerial biomass and malate:sulfate in leaf, a quadrant methodology was used as described by Cate and Nelson (1965). According to this methodology, four possible quadrants are defined: 1) S sufficient and correctly diagnosed plants (yield > 90% of maximum and ratios < critical level), 2) S sufficient plants with incorrect diagnosis (yield > 90% of maximum and ratios > critical level), 3) deficient plants and incorrect diagnosis (yield < 90% of maximum and ratios < critical level) and 4) deficient plants and correct diagnosis (yield < 90% of maximum and ratios > critical level). Quadrants 1 and 4 are considered positive while quadrants 2 and 3 are considered negative.

Lastly, a validation of the proposed thresholds for the grain analysis methodology was realized with information from other ten fertilization experiments conducted in the area along 2003, 2004, 2005, 2007 and 2008 (2, 2, 2, 1 y 3 experiments, respectively). Furthermore, in the 3 experiments carried out in 2008, aerial biomass sampling was done at stages Z24, Z31 and Z39, and N and S concentration was determined by the Dumas method. This information was used to validate the proposed thresholds for N:S ratio in aerial biomass.

Data Analysis

The PROC UNIVARIATE procedure was used to test the normality assumptions of the evaluated variables, besides analyzing residue distribution (SAS Institute, Cary, NC, USA). On the first year, variance analysis was done using a PROC MIXED procedure included in the Statistical Analysis System program protocols (SAS Institute). This model considers sites and treatments as fixed effects. The significance level was 5%. In the second year, variance analysis was done through the GLM procedure (SAS Institute). When differences between treatments were significant, a least significant difference (LSD) test was used, with a significant level of 5% (SAS Institute). Some variables were evaluated by regression and slopes were compared through the parallelism and coincidence test using the PROC REG procedure (SAS Institute).

RESULTS AND DISCUSSION

Environmental Characterization of Experiments

In Sites 1, 3, 4, and 6 water availability during growth season was adequate to meet crop needs (380–420 mm approximately); however, at Sites 2 and 5 only 314 and 266 mm of precipitation was recorded. This might have limited wheat yielding as the lower rainfalls took place along the critical period. On the other hand, at Sites 5 and 6 the average temperature observed for November and December was 4 and 2°C higher than the normal average, respectively (INTA Balcarce, 2009), which could have decreased grain quantity and weight.

Grain Yield and Its Components

Average crop yield at Sites 1, 2, 3, 4, 5, and 6 was 6675, 3657, 5565, 3775, 4264, and 3893 kg ha^{-1}, respectively. At Sites 3 and 4, significant yield differences were determined by applying S, with a bigger response to S higher rates of 37 y 34%, respectively (Table 2). The higher response to S addition can be explained by a low S availability at sowing and low soil organic matter (OM) content (Table 1), typical of land plots from southeast Buenos Aires with an extended cropping history (Studdert and Echeverría, 2000a). Beaton and Soper (1986), determined a critical S availability in soil of 36 kg ha^{-1} (0–60 cm), which partially explains the results of our experiment. Besides, for the same region, Reussi Calvo et al. (2006a) obtained similar soil critical levels for wheat crops. Even when in Site 1 S availability at sow was similar to other sites, higher OM content would explain the lack of response to S addition (Tables 1 and 2). At Sites 2 and 5, yield increase by S addition could not be determined because of the low water availability during the crop critical period. The lack of response to S addition at Site 6 can be related to the effect of wheat as the preceding crop and unfavorable temperatures by the end of the growing season (Table 3). Considering the low residue quality of grasses compared to legumes and sunflower (Studdert and Echeverría, 2000b), wheat as the preceding crop might have decreased the contribution of N by mineralization, which might have limited the response to S addition.

TABLE 2 Grain yield, its components and nitrogen and sulfur concentration in grain at the first years

Site	Treatment	Grain yield (kg ha^{-1})	GN (m^{-2})	SN (m^{-2})	GW (g)	Grain N (%)	Grain S (%)
1	0 S	6708a	17170a	767a	34a	2.50a	0.18a
	5 S	6630a	17581a	786a	32a	2.50a	0.18a
	10 S	6572a	17906a	752a	32a	2.50a	0.19a
	20 S	6788a	18301a	784a	32a	2.59a	0.18a
2	0 S	3545a	9658a	504b	32a	2.73a	0.16b
	5 S	3607a	9964a	587ab	31a	2.70a	0.18ab
	10 S	3619a	9736a	621a	32a	2.83a	0.18a
	20 S	3858a	10511a	597a	32a	2.74a	0.19a
3	0 S	4396b	10367b	419b	36ab	2.46a	0.12c
	5 S	5885a	13765a	456ab	37a	2.42a	0.15b
	10 S	5966a	14828a	525ab	35b	2.36a	0.15ab
	20 S	6013a	14975a	561a	35b	2.33a	0.16a
4	0 S	3099b	8984b	627a	30ab	2.78a	0.19a
	5 S	3901a	10869a	624a	31a	2.78a	0.19a
	10 S	3949a	11671a	628a	29ab	2.82a	0.20a
	20 S	4149a	12389a	678a	29b	2.88a	0.20a

GN = grain number; SN = spike number; GW = grain weight.

In each column, the same letter indicates no significant differences between the means within site ($P < 0.05$).

This N availability reduction was reflected in the low number of spikes m^{-2} and low yield even at N high rates (Table 3).

In previous works, for similar conditions as those in this experience, an 11% in yield increase was reached by adding S (Reussi Calvo et al., 2006b; Salvagiotti and Miralles, 2008). However, response values as determined by this work were within the wide range reported for winter wheat (from 4 to 81%) by Zhao et al. (2002). This high variability in S addition response is related to differences in soil type, in crops potential yields, and the contribution of atmosphere S (McGrath et al., 2002).

In general terms, at sites without response to S addition (1, 2, 5 and 6), no significant differences were determined in any of the yield components as effect of S addition (Table 2 and 3). Only in Site 2 the number of spikes from S high rates treatments was higher than 0 treatments. On the contrary, for sites with response (3 and 4), increases in grain number and decrease in their weight by S addition was determined (Table 2). Other authors determined at adequate N availability conditions increases in the number of grains with S addition (Salvagiotti and Miralles, 2008). Decrease in grain weight can be explained by a dilution effect as a result of the higher yield of fertilized treatments (Table 2). Furthermore, in marginal S deficiency conditions, Zhao et al. (1997a) determined reductions in the weight of 1000 grains and in the specific weight of grains with S addition. Lastly, in Site 3 significant increase in the number of spikes was determined as a result of fertilization with S (Table 2). Inal et al. (2003) and Salvagiotti and Miralles (2008),

TABLE 3 Grain yield, its components and nitrogen and sulfur concentration in grain during the second year

Site	SR ($kg\ ha^{-1}$)	NR ($kg\ ha^{-1}$)	Grain yield	GN (m^{-2})	SNE (m^{-2})	GW (g)	Grain N (%)	Grain S (%)
5		0 N	3151	8522	417	32	1.53	0.13
	0S	90 N	4128	11680	483	30	1.61	0.14
		130 N	4567	12697	528	31	1.82	0.14
		170 N	4739	12915	560	32	1.92	0.15
		0 N	3760	10190	426	32	1.53	0.14
	40S	90 N	4243	11726	457	31	1.65	0.15
		130 N	4491	12196	505	32	1.75	0.15
		170 N	5035	13857	551	31	1.82	0.16
Means NR		0 N	3456c	9356c	421c	32	1.53d	0.14b
		90 N	4185b	11703b	470bc	31	1.63c	0.14b
		130 N	4529ab	12446ab	516ab	31	1.78b	0.15b
		170 N	4887a	13386a	556a	31	1.87a	0.16a
Means SR		0 S	4146	11453	497	31	1.72	0.14b
		40 S	4382	11992	485	31	1.69	0.15a
LSD	NR		*	*	*	ns	*	*
LSD	SR		ns	ns	ns	ns	ns	*
LSD	NR*SR		ns	ns	ns	ns	ns	ns
6		0 N	2003	4655	260	37	2.23	0.18
	0S	90 N	3673	8198	350	39	2.16	0.19
		130 N	4547	10549	396	37	2.25	0.20
		170 N	5050	11550	415	38	2.40	0.22
		0 N	2334	5366	255	37	2.10	0.19
	40S	90 N	4233	9359	357	39	2.02	0.20
		130 N	4478	10326	392	37	2.23	0.21
		170 N	4829	11367	407	37	2.38	0.23
Means NR		0 N	2168c	5011c	258b	37b	2.17bc	0.19c
		90 N	3953b	8778b	354a	39a	2.09c	0.19c
		130 N	4513ab	10437ab	394a	37b	2.24b	0.21b
		170 N	4939a	11458a	411a	37ab	2.39a	0.22a
Means SR		0 S	3818	8738	356	38	2.27	0.20b
		40 S	3968	9104	353	38	2.19	0.21a
LSD	NR		*	*	*	*	*	*
LSD	SR		ns	ns	ns	ns	ns	*
LSD	NR*SR		ns	ns	ns	ns	ns	ns

GN = grain number; SN = spike number; GW = grain weight. NR = nitrogen rates; SR = sulfur rates. * = significant difference at 5%, ns = no significant. Means within a column followed by the same letter are not significantly different at the 5% (LSD test).

obtained significant differences in the number of spikes by fertilization with S, but those differences were lower than those determined in this work.

On the second year, no significant interaction on crop yield or in any of its components was determined by N and S addition (Table 3). On the other hand, wheat yield, the number of grain m^{-2}, and spikes m^{-2} increased significantly by effect of N addition (Table 3). According to Abbate et al. (1994), the number of grains is the main yield component affected in low N availability conditions because of leaf area reduction and the conversion efficiency of intercepted radiation. Response to N addition have been frequently reported for wheat (Melaj et al., 2003; Salvagiotti and Miralles, 2008; Barbieri et al., 2008).

Nitrogen and Sulfur Concentrations in Grain

The average concentration of N in grain at Sites 1, 2, 3, 4, and 6 was above of critical level (2%) determined for wheat grain by Goos et al. (1982). However, in Site 5, N contents in grain were lower than the aforementioned critical level (Table 3). The low water availability during crop cycle (266 mm) could have limited the soil N mineralization and N absorption. Xu et al. (2005) determined increases in N accumulation with water availability increase. Similar results were obtained by Ercoli et al. (2007) for hard wheat.

On the other hand, no significant interaction was determined on N concentration in grain as an effect of N and S levels (Table 3). Besides, in none of the experimental sites were found significant increases in grain N concentration by S addition (Tables 2 and 3). Similar results were obtained in wheat by Randall et al. (1981), Hawkesford et al. (2002), Inal et al. (2003) and Reussi Calvo et al. (2006b) and in barley by Zhao et al. (2006). However, N concentration in grain increased because of the increase of N availability (Table 3). These results are similar to those determined in wheat by Melaj et al. (2003), Subedi et al. (2007) and Barbieri et al. (2008).

Grain S concentration in every treatment except the 0S treatment at Site 3, was higher than the 0.12% of total S, value considered as critical level by Randall et al. (1981) (Table 2 and 3). No significant interaction on S concentration in grain as effect of N and S addition was determined (Table 3). At Sites 2, 3, 5 and 6, significant increase in S concentration was determined as a result of S addition (Table 2 and 3). Several works, carried on different crops, determined increase in grain S concentration by S fertilization (Moss et al., 1981; Zhao et al., 1999b, 2006; Prystupa et al., 2006). However, the lack of response at Site 1 and particularly at Site 4, which showed yield increase by S addition, could be explained by the late contribution of S through mineralization, thus enabling right S concentrations at crop. Under controlled conditions, Monaghan et al. (1999) determined that 50% of S accumulated in wheat grain comes from its postanthesis uptake.

Lastly, on the second year sites, significant differences were observed in S concentration in grain as an effect of N application (Table 3). The higher S content was determined at the maximum N rates. These results confirm what was determined by Rasmussen et al. (1975) and Randall et al. (1981), who concluded that S concentration in grain does not depend only on S availability but also on N availability. More recent works have reached increases in S concentration in grain as a result of N rates increase (Flaetea et al., 2005; Lerner et al., 2006).

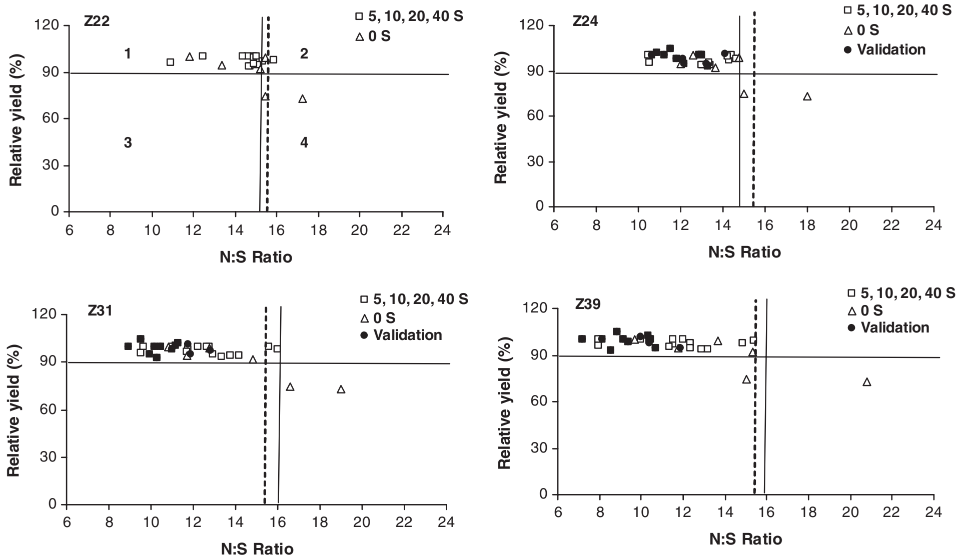

FIGURE 1 Relationship between relative yield and total N:S ratio in spring red wheat for S rates. The horizontal line describes the 90% maximum grain yield. Vertical continue line correspond to critical N:S ratio of 15.2:1, 14.8:1, 16:1 and 16:1 for Z22, Z24, Z31 and Z39, respectively. Vertical discontinue line correspond a critical total N:S ratio of 15.5:1, value proposed in this experience. 1 y 4 = positive quadrants, 2 y 3 = negative quadrants. (●) = independent data set (2008).

Sulfur Diagnosis: Plant Analysis

The relationship between relative yield and N:S ratio in aerial biomass is shown in Figure 1. Through positive quadrants points counting (Cate and Nelson, 1965), 90, 100, 100 and 95% of samples were correctly diagnosed, for stages Z22, Z24, Z31, and Z39, respectively (Figure 1). These values are slightly above those obtained by Blake Kalff et al. (2000 and 2004) for winter wheat. On the other hand, a critical N:S ratio of 15.2:1 was determined for Z22, 14.8:1 for Z24, and 16:1 for Z31 and Z39 (Figure 1). This means that even when the critical N:S ratio was slightly affected by the sampling moment, differences were minimal, thus a critical average N:S ratio of 15.5:1 could be used for the period extending from the beginning of tillering through the end of stem elongation. The definition of this unique critical N:S ratio was done in a similar way to Blake Kalff et al. (2000). Similar critical ratios have been reported by other authors (Spencer and Freney, 1980, Blake Kalff et al., 2000). Furthermore, when this critical N:S ratio (15.5:1) was validated with information from the three 2008 experiments, 100% of samples were correctly diagnosed (Figure 1). These results confirm the 15.5:1 threshold to diagnose S deficiency in spring wheat.

For malate:sulfate ratio, 60, 60, 45, and 35% of the samples were correctly diagnosed for stages Z22 and Z24, Z31, and Z39, respectively (Figure 2). These results were obtained using 1.5 as critical level, according to Blake

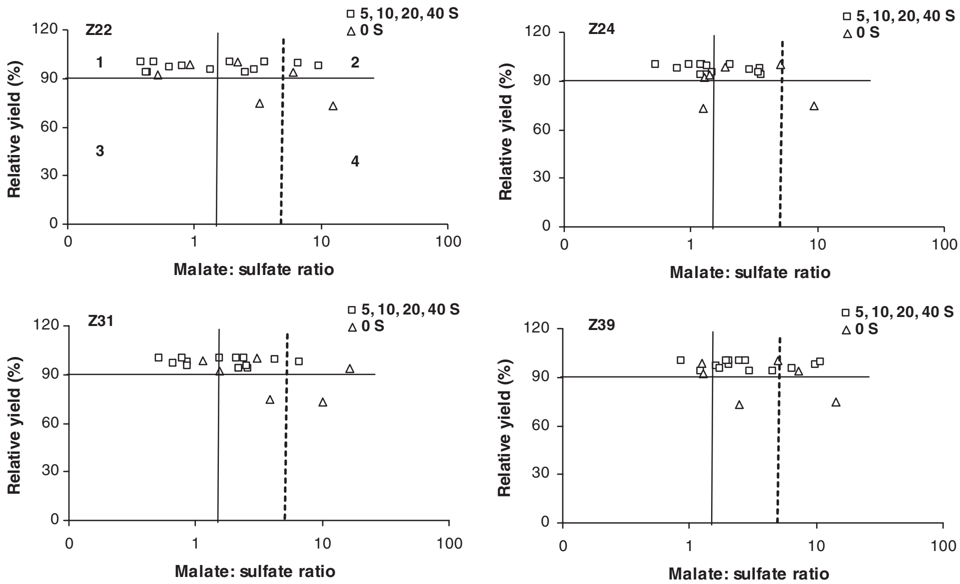

FIGURE 2 Relationship between relative yield and malate: sulfate ratio in spring red wheat for S rates. The horizontal line describe the 90% maximum grain yield. Vertical continue line correspond a malate: sulfate ratio of 1.5, value reported by Blake Kalff et al. (2004) as thresholds of plant S deficiency. Vertical discontinue line correspond a malate: sulfate ratio of 6, value proposed in this experience. 1 y 4 = positive quadrants, 2 y 3 = negative quadrants.

Kalff et al. (2004) for winter wheat. The number of correctly diagnosed cases is below the 76% determined by those authors. However, in a recent work done over 19 places from England and Scotland, Carver (2005) determined 54% of samples correctly diagnosed, close to the value determined in our experience. Besides, malate:sulfate ratio had an average of 49 and 1% of samples diagnosed incorrectly as deficient and sufficient, respectively (Quadrant 2 and 3, Figure 2). Considering the same quadrants, Blake Kalff et al. (2004), determined only 20 and 4% of samples were incorrectly diagnosed, while the Carver (2005) reported that 42 and 7% of samples were incorrectly diagnosed. One possible explanation of the high rate of incorrectly diagnosed points in Quadrant 2 might be the occurrence of temporary S deficiencies, reversed lately by sulfate contribution from sub-surface horizons and/or mineralization as the wheat growth season progresses. Another possible explanation could be the positive effect of N on malate concentration in leaf. Reussi Calvo (2009) determined increments in malate concentration in leaf by increases in N availability. This generates high malate:sulfate ratios, which could increase the number of cases incorrectly diagnosed (Quadrant 2, Figure 2). Anyway, it is important to mention that this type of error (Quadrant 2) would produce overfertilization with S, while the samples at Quadrant 3 would indicate non fertilization with S when it is necessary. From a profitability point of view, it is usually accepted that the Quadrant

3 mistake is more detrimental than the Quadrant 2 mistake, while from an environmental point of view and in the long term, it is the other way around.

Lastly, an aspect to be considered is that probably the critical malate:sulfate ratio for spring wheat might be higher than for winter wheat because of the higher potential yielding of the latter. When in this experience the critical threshold was modified in order to minimize the number of points in negative quadrants, a critical level of 6 improved the predictive capacity of the methodology as consequently the number of samples correctly diagnosed were 80, 95, 85 y 75% for stages Z22 and Z24, Z31 and Z39, respectively (Figure 2).

Sulfur Diagnosis: Grain Analysis

Considering the critical thresholds established by Randall et al. (1981), the only grain sample with S deficiency would be for 0S treatment at Site 3 (Figure 3), which confirms what was determined in this experience (Table 2). However, 0S treatment at Site 4 was diagnostic as sufficient (Figure 3) when in reality it was not (Table 2). Furthermore, according to this methodology, the most of situations were with N deficiency, but N concentration determined at Sites 1, 2, 3, 4, and 6 was above of grain N critical threshold (2%) proposed by Goos et al. (1982) (Table 2 and 3). Other authors, at similar conditions as this experience, determined a high percentage of samples diagnosed as N deficient, but concentration of the aforementioned nutrient in grain was the right one (Reussi Calvo et al., 2006a, b). Nevertheless, N deficiency in grain at Site 5 was correctly diagnosed (Figure 3 and Table 3).

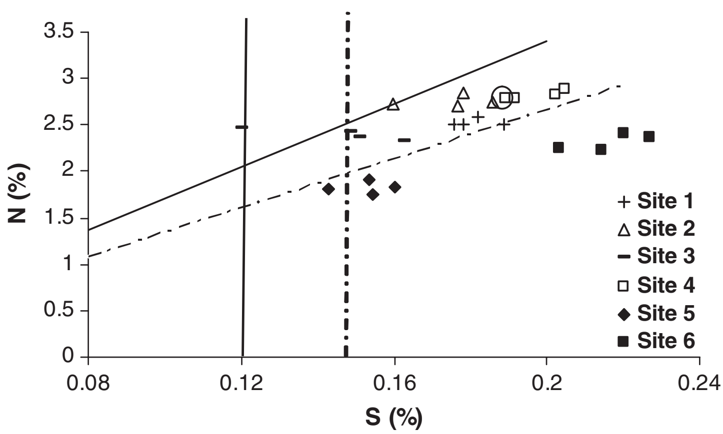

FIGURE 3 Relationship between total N and S concentration of wheat grain. Vertical and oblique continue lines corresponds to 0.12% of S concentration and a N:S ratio of 17:1 (total N and S), values reported by Randall et al. (1981) as thresholds of grain S deficiency. Vertical and oblique discontinue lines corresponds to 0.15% of total S concentration and a N:S ratio of 13.3:1, values were determinate in this experience. (•) = 0 S for Site 4.

In a work conducted at several places in Hungary, Gyori (2005) determined a critical concentration of S in grain of 0.15%, which is higher than the obtained by Randall et al. (1981). Besides, Carver (2005) using a critical N:S ratio of 17:1 determined only 42% of samples correctly diagnosed when analyzing sites with S deficiencies. Considering the critical concentration of S in grain proposed by Gyori (2005) and a 2% N critical concentration (Goos et al., 1982), the critical N:S ratio in grain was re-estimated, resulting in a critical value of 13.3:1. Even when this ratio is lower than the one determined by Randall et al. (1981), it is close to the one obtained by Bergmann (1992), which was 14.8:1. Figure 3 shows the ratio between total N concentration and total S concentration in grain considering these new critical levels. For the first year sites, a consistent improvement in the predictive capacity of this grain analysis methodology can be observed. However, at Site 6 this methodology keeps showing N deficiency when there was not (Figure 3 and Table 3). This could be explained by high S concentrations in grain (Table 3), which produce low N:S ratios and cause error in the methodology. Several works have determined N:S ratio in grain decreases when S availability increases (Zhao et al., 1997b; Hawkesford et al., 2002; Inal et al., 2003). On the other hand, 0S treatment at Site 4 still shows S sufficiency (Figure 3). This could be explained by a late S contribution by mineralization, which increased S concentrations in grain but does not prevent wheat yield loss. Besides, it is important to underline that at Site 4, wheat was grown under LC and with sunflower as preceding crop, favorable conditions for S mineralization. Moreover, when the new critical N:S ratio and S total values were validated with independent data set (10 experiments), the number of cases correctly diagnosed were 70% (Figure 4).

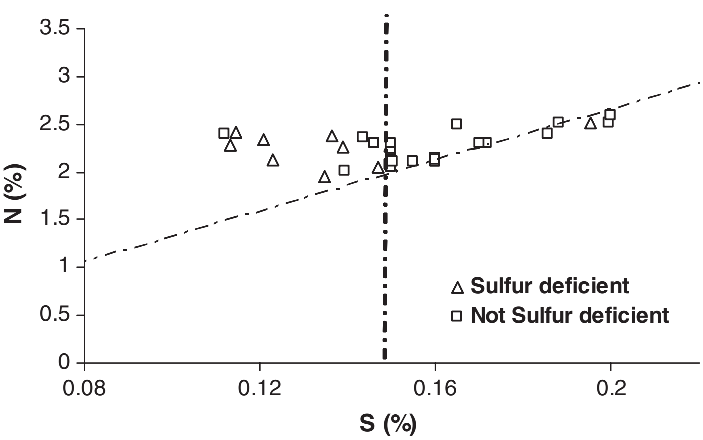

FIGURE 4 Relationship between total N and S concentration of wheat grain for at sites without response and with response in yield for sulphur application. Vertical and oblique discontinue lines corresponds to 0.15% of total S concentration and a N:S ratio of 13.3:1, values were determinate in this experience. Independent data set (2003, 2004, 2005, 2007 and 2008).

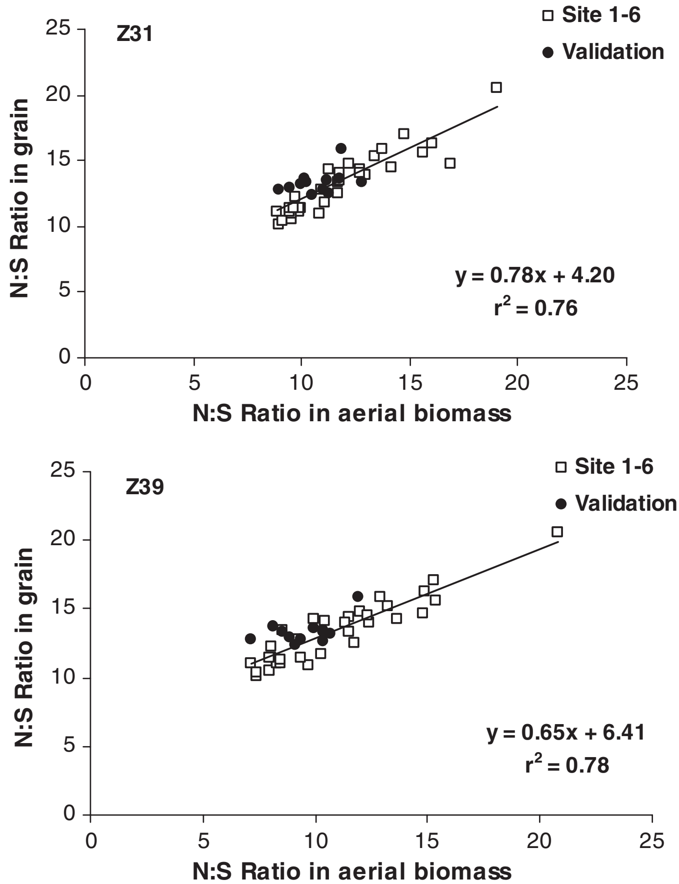

FIGURE 5 Relationship between total N:S ratio in grain and total N:S ratio in aerial biomass for Z31 and Z39 growth stages. (•) = independent data set (2003, 2004, 2005, 2007 and 2008).

Lastly, Figure 5 shows the association between N:S ratio in grain and N:S ratio in aerial biomass for stages Z31 and Z39. A close linear relation among both variables was determined (Figure 5). Besides, this relation was validated using the data from the three 2008 experimental sites. These results allow us to make N:S ratio in grain estimations from N:S ratio in plant, in order to monitor the aforementioned ratio and, if necessary, improve through later applications of N or S. In this way, future imbalances of these nutrients in wheat grain proteins might be corrected, thus avoiding quality loss. These ratios should be confirmed in different edaphoclimatic conditions.

CONCLUSIONS

Although the predictive capacity of the malate:sulfate ratio in leaf improved with the increase of the critical level from 1.5 to 6, this would increase the possibility of mistake in sites with S response. However, N:S ratio in aerial biomass was a more reliable indicator than malate:sulfate ratio in leaf in order to diagnose S deficiency in wheat. On the other hand, the use of total S concentration and N:S ratio in grain as indicator of the S availability for the crop became a reliable tool when the thresholds proposed by Randall et al. (1981) were modified. Two new thresholds (0.15% total S and total N:S ratio 13.3:1) showed a good behavior when validated with independent data set.

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